The present collection also contains several specimens of the fish *Carrionellus diu-mortuus* Ivor White from the nudo of Cajanuma at the southern end of the Loja Basin.

All of these forms, with the exception of the new species of *Pithecolobium* and the *Ruprechtia*, are common elements in the flora of the Loja Basin, and the deposits of these inter-montane basins in the Ecuadorian Andes are evidently all of approximately the same age.

Recently I described several occurrences of fresh water mollusks and land plants from the Cuenca Basin in Ecuador.[4] These came from near the town of Biblian in the Azogues valley, so that there is now definite evidence of the presence of similar late Tertiary continental deposits of probably fluviatile palustrine and lacustrine character, and possibly eolian as well, largely made up of volcanic ash, over a north and south distance of upwards of 150 miles. It seems very probable that similar fossiliferous deposits of approximately the same age may be expected in the other inter-Andean basins north of the Cuenca Basin.

Malacatos has a present altitude of 5187 feet which is from 1800 to 2100 ft. lower than the plant bearing outcrops in the Loja Basin and about 2800 ft. lower than the similar outcrops in the Cuenca Basin. At the present time the climate at Loja and Cuenca is arid temperate, while that at Malacatos is subtropical. In all cases the fossil plants are mesophytic tropical types and the evidence is clear that there has been a considerable amount of vertical uplift since these deposits were laid down. Whether or not their present altitude is to be ascribed to differential uplift or to deposition at originally different levels can not be stated, although it seems clear that all occurred at the same physiographic stage in the geological history of the region.

[4] BERRY, EDWARD W. This JOURNAL 24: 184–186. 1934.

ZOOLOGY.—*Life history of* Longistriata musculi, *a nematode parasitic in mice.*[1] BENJAMIN SCHWARTZ and JOSEPH E. ALICATA, Bureau of Animal Industry.

This paper contains a brief account and discussion of the life history of a trichostrongyle, *Longistriata musculi*, parasitic in the intestine of the mouse, *Mus musculus*, and readily reared to fertile maturity in white mice. In addition to the conventional account of the life history, the writers have included in this paper information on the course of infection, including a consideration of such problems as the

[1] Received December 10, 1934.

egg production, susceptibility of the host to reinfection following the apparent termination of egg production, and a discussion of the results obtained.

METHODS USED

Live infested mice were shipped to Washington, D. C., from Jeanerette, Louisiana. The feces of these animals were mixed with moist animal charcoal, and the mixture was placed on moist filter paper in covered petri dishes. The infective larvae migrated to the edges of the filter paper which were turned up at right angles to the bottom of the glass dishes. The larvae were readily detected along the edges of the filter paper, usually in clusters, adhering to the paper by their tails and waving the anterior portions of their body. By cutting off portions of the filter paper on which larvae had accumulated and placing the bits of paper in a glass dish containing a small quantity of water, the larvae could be counted readily when comparatively few were present. When large numbers of larvae were obtained in this manner they were counted by the dilution method.

In studying the development of the free-living stages, the writers isolated single eggs with the aid of a capillary pipette, and placed each egg in a drop of very dilute fecal emulsion in a small stender dish having an inside diameter of 20 mm. The dishes were kept in a moist glass chamber containing several layers of wet filter paper. The individual dishes were taken out of the moist chamber as often as necessary and examined microscopically to ascertain the progress in development.

The individual mice were kept and fed in large battery jars, a folded paper hand towel being used as bedding. The animals were fed on oats, and this was supplemented by cabbage twice a week. The feces, bedding and remnants of food particles were removed daily, and the jars were scalded with hot water and then dried. This procedure precluded the possibility of extraneous infection.

In experimental percutaneous infections, the infective larvae in a small quantity of water were placed on various portions of the skin of white mice anesthetized with ether, the mice being kept under anesthesia until the water containing the larvae had evaporated. The larvae were placed on portions of the skin from which the hair had been clipped or shaved. Larvae were introduced into the mouth in a small quantity of water with the aid of a pipette. The lungs, liver, portions of the wall of the alimentary canal and other organs were examined post mortem for larvae with the aid of the Baermann ap-

paratus and in press preparations. The heart's blood and other fluids of the body were removed to glass slides with the aid of capillary pipettes, after being diluted with physiologic saline and examined for larvae. Mature worms were obtained from the lumen of the intestine by slitting the wall of this organ in a glass dish containing physiologic saline and removing the worms from the solution as well as from the lining of the intestine.

The Stoll dilution technique was used in making egg counts. The total fecal output for 24 hours of the mice involved in this investigation in no case exceeded 0.22 gms., and usually weighed about 0.2 gms.; in a few cases the weight was as low as 0.05 gms. In making fecal dilutions for the counts, practically the entire fecal sample was used in nearly all cases. For the purpose of ascertaining the presence of eggs, the salt flotation technique was used.

PREPARASITIC DEVELOPMENT

The segmented eggs eliminated with the feces of infested mice hatched in about 24 hours in laboratory cultures maintained at a temperature of 24°C. The newly hatched larva feeds almost continuously and grows considerably during the feeding period which lasts about 4 days during the summer months. The molting larva is encased in a sheath, the cuticle of the first-stage larva, which apparently is not discarded in water. On solid culture media, consisting of moist animal charcoal to which mouse feces have been added, the sheath is discarded. The exsheathed larva is infective to mice, and is morphologically and physiologically identical with the third-stage larva of other strongyles; as will be shown in connection with its morphology and in the discussion, it should be regarded as corresponding to a third-stage rather than a second-stage larva, on the assumption that the first molt has been suppressed.

EXPERIMENTAL INFECTIONS THROUGH THE MOUTH

Experiment 1. Each of two mice (nos. 1 and 2) was fed 500 infective larvae. Five days after the experimental feeding, the feces of these mice were still free of eggs; 7 days after the experimental feeding a few eggs of *L. musculi* were found in the feces of mouse no. 1 and numerous eggs were found in the feces of mouse no. 2.

Experiment 2. Mouse no. 3 was given 6 feedings of 100 larvae each as follows: May 23, 1 P.M.; May 24, 9 A.M.; May 25, 9 A.M., 4 P.M. and 9 P.M.; May 26, 9 A.M. The mouse was killed on May 26,

11:30 A.M., 70½ hours after the initial feeding and 2½ hours after the last feeding. Post-mortem examination for worms yielded the following results:

Thirty-five larvae showing no increase in size and no progress in development beyond those of the infective larvae, were found in the stomach; in the small intestine there were present 143 larvae, some showing no evidence of growth beyond that of the infective larva, others showing an increase in size, and some showing early signs of the first parasitic molt, in addition to 140 preadult worms corresponding morphologically to fourth-stage larvae of other strongyles; of these worms 63 were males and 77 were females. The large intestine contained 9 living infective larvae. The liver, lungs and heart's blood were examined for larvae with negative results.

Experiment 3. Mouse no. 4 was given 2 feedings of 100 larvae each on May 29, 2:30 P.M., and May 31, 2:30 P.M. This mouse died some time between 4:30 P.M., May 31, and 9 A.M., June 1. Post-mortem examination revealed 30 larvae in the stomach showing no evidence of growth beyond that attained by the infective larvae, 80 worms in the small intestine, of which 49 (18 males and 31 females) were in the preadult stage and 31 were in the infective stage. No larvae were found in the liver and lungs.

Experiment 4. Mouse no. 5 was given 200 infective larvae on June 2. On June 7, 5 days after experimental feeding, this mouse was killed and examined for evidence of infestation with the following results:

The small intestine contained 32 worms of which 22 (15 males and 7 females) were in the preadult stage, but were already in the third or final ecdysis, while the remaining 10 worms (6 males and 4 females) were in the final, or adult, stage, having discarded the sheath of the last molt before the host animal was killed. The females did not as yet contain eggs in the uteri. No worms were found elsewhere in the alimentary canal. The lungs were free of worms.

It is evident from these data that the entry of *Longistriata musculi* larvae through the oral route not only leads to the development of these worms to fertile maturity, as evidenced by the appearance of eggs in the feces of the experimental host animal on the seventh day following the administration of the larvae (experiment 1), but that the entire development takes place in the small intestine, as shown in experiments 2, 3 and 4. All the developmental stages, beginning with those indistinguishable from the infective stage, through the various growth changes in that stage, the first parasitic ecdysis, the preadult

stage, which follows the casting off of the sheath, growth changes during the preadult stage, the second parasitic ecdysis, and adult or final stage which follows the final exsheathing, were found in the small intestine. No evidence was found of a migration of the larvae from the alimentary canal to the liver or lungs. *Longistriata musculi* is, therefore, capable of achieving its full development in the intestine following the ingestion of the infective larvae. The latter reach the stomach first, and in this organ some of them, and perhaps all of them, linger for a while and then pass into the small intestine where sexual maturity is attained following growth and development accompanied by 2 molts. Preadult worms were already present in experimentally infected mice about 48 hours after experimental feeding, and adult worms, not yet fully grown, were found 5 days after experimental feeding. The entire parasitic development, commencing with the ingestion of infective larvae and ending in egg-laying maturity, was completed in 7 days.

EXPERIMENTAL INFECTIONS THROUGH THE SKIN

Mice were exposed to experimental infections through the skin with a view to (1) determining whether the skin is a suitable portal of entry of *Longistriata musculi* larvae into the body of the rodent host; (2) tracing the course of migration of the parasites from the skin to the small intestine; and (3) ascertaining the precise locations in the body where the development of the larvae is resumed after being suspended following the preparasitic molt. The results of experimental percutaneous infections involving 17 mice, examined at various intervals following the exposure of the skin to infective larvae, the intervals ranging from $\frac{1}{2}$ hour to 7 days after infection and corresponding to the periods during which migration, growth and development take place, are summarized in table 1.

An examination of the data presented in table 1 shows among other things (1) that the larvae which were placed on the intact skin actually penetrated this tissue and that some of them were still present in the skin layers 4 hours after having been placed on the surface; (2) that at least one larva was found in the stomach as early as one hour after the exposure of the skin to larvae and that fairly large numbers of larvae were found in the stomach 3, $4\frac{1}{2}$ and 6 hours, respectively, following the placing of the larvae on the skin; (3) that the larvae were found in the stomach before they were seen in the small intestine or that many more were present in the stomach than in the small intestine up to 6 hours following skin infection; (4) that

some larvae reached the small intestine as early as 3 hours after they had been placed on the skin and that 10 hours after skin exposure the number of larvae which were present in the intestine was in excess of those present in the stomach; (5) that 24 hours following exposure of the skin to larvae, the latter were localized exclusively in the small intestine, in which organ they continued their development; (6) that preadult worms were present in the intestine about 48 hours

TABLE 1.—Results of Percutaneous Infections of 17 Mice

Mouse Number	No. of larvae placed on skin	Duration of experiment	Post-mortem results[a]
6	150	1 hour	20 larvae in skin and 1 in stomach; all in infective stage
7	500	2 hours	24 larvae in skin
8	800[c]	1–3 hours	26 larvae in stomach and 5 in intestine; all in infective stage
9	600[b]	½–3½ hours	22 larvae in skin; all in infective stage
10	800[c]	½–4 hours	1 larva in lungs, 2 in esophagus, 15 in stomach, 7 in intestine; all in infective stage
11	1,000	4 hours	4 larvae in skin; all in infective stage
12	1,000	4 hours	Negative
13	1,000	4½ hours	6 larvae in stomach; all in infective stage
14	1,000	6 hours	76 larvae in stomach; all in infective stage
15	150	10 hours	11 larvae in stomach, 27 in intestine; all in infective stage
16	1,000	10 hours	34 larvae in stomach, 94 larvae in intestine; all in infective stage
17	1,000	24 hours	228 infective larvae
18	1,000	24 hours	109 larvae in intestine; stage not noted
19	1,000	48 hours	103 preadult worms in intestine
20	1,000	72 hours	72 preadult worms in intestine
21	200	120 hours	38 worms in intestine; 11 males and 11 females in final stage, and 9 males and 7 females in preadult stage
22	500	7 days	86 fully developed worms (41 males and 45 females in intestine)

[a] Larvae placed on skin as follows: 400 at 11 A.M.; 200 at noon; 200 at 1 P.M. Mouse killed at 2 P.M.
[b] Four consecutive infections of 150 larvae each at intervals of one hour. Mouse killed 30 minutes after final exposure to infections.
[c] Four consecutive infections of 200 larvae each at one-hour intervals. Mouse killed 30 minutes after final exposure to infection.

after skin exposure; (7) and that 5 days after experimental infection the majority of the worms were already in the final (adult) stage, and that 7 days after infection all the worms present in the intestine had attained the adult stage.

Although the data on mouse no. 10 appear to indicate that the path followed by the larvae from the skin to the intestine was the route usually followed by skin-penetrating nematodes, namely from skin to the lungs by way of the circulation and from the lungs to the intestine by upward migration in the bronchioles, bronchi and trachea, and thence back to the alimentary canal, the post-mortem data on the re-

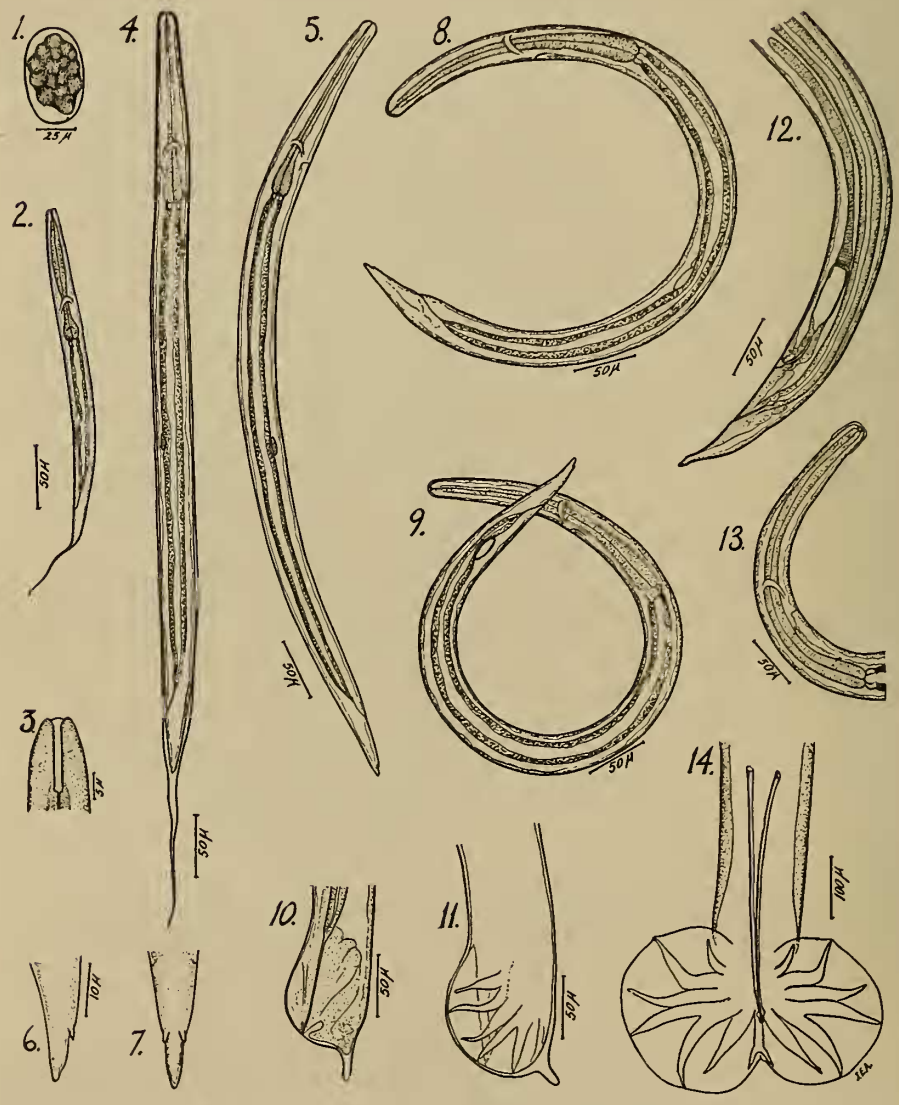

Fig. 1–14.—Stages in the development of *Longistriata musculi*. Fig. 1.—Egg from fresh feces. Fig. 2.—Newly hatched larva. Fig. 3.—Anterior end of preinfective larva. Fig. 4.—Preinfective molting larva. Fig. 5.—Infective larva. Fig. 6.—Tail of infective larva (lateral view). Fig. 7.—Tail of infective larva (ventral view). Fig. 8.—Male larva showing the beginning of the first parasitic molt. Fig. 9.—Female larva showing the beginning of the first parasitic molt. Fig. 10.—Posterior portion of preadult male, 3 days after experimental infection. Fig. 11.—Posterior portion of preadult male in the final molt, 5 days after experimental infection. Fig. 12.—Posterior portion of preadult female. Fig. 13.—Anterior portion of preadult male. Fig. 14.—Bursa of young adult male, 5 days after experimental infection.

maining mice given in table 1 do not support this assumption, despite the evidence that the larvae reached the stomach before they appeared in the intestine in some of the experimental infections. Careful examination of the hearts' blood, the fluid of the peritoneal and thoracic cavities, the lymph glands, lungs, liver, spleen, pancreas, kidneys, and other organs and tissues in which larvae might be present if they were carried in the circulation, yielded consistently negative results in all cases in which such examinations were made, and practically all the mice involved in this investigation were examined with a view to determining the probable path of migration. Aside from this negative helminthological evidence, no lesions suggestive of lung invasion by nematode larvae were noted in any of the mice involved in this investigation. There was a complete absence of petechial and ecchymotic spots in the lungs, lesions usually associated with the invasion of the lungs by nematode larvae.

While the possibility of a direct migration to the alimentary canal through the tissues and cavities of the body must be considered as an alternative to migration through the lungs, the available evidence, especially the failure to find larvae in press preparations of the wall of the stomach and small intestine, lends no support to this possible migratory route. The question of the path followed by the larvae of *Longistriata* from the skin to the alimentary canal must be left open for the time being.

MORPHOLOGICAL ASPECTS OF DEVELOPMENT

The outstanding morphological features in the development of *L. musculi* are shown in the illustrations (figs. 1–14). The brief descriptions which follow help to clarify the illustrations.

Egg.—The egg (fig. 1) has a morphology characteristic of other trichostrongyle eggs; it is 61 to 68μ long by about 38μ wide, elliptical in shape, thin shelled, and segmented when found in fairly fresh feces.

Preinfective larva.—This larva (fig. 2) resembles those of other members of the family Trichostrongylidae. It is slender, cylindrical, tapering slightly anteriorly and more so posteriorly, and is provided with a long filamentous tail. The newly hatched larva is from about 296 to 311μ long by 17μ wide. The mouth opening leads into a cylindrical buccal cavity or pharynx (fig. 3) about 15μ long; the esophagus is characteristically rhabditiform, 91 to 95μ long, its bulb being provided with the usual Y-shaped valve; the intestine, about 120μ long is followed by a short rectum. The nerve ring is about 65 to 79μ, and

the genital primordium 152 to 167μ, respectively, from the anterior extremity. The tail is 60 to 68μ long.

The first preinfective larva grows considerably, attaining a length of 750μ, including the long filamentous tail. At this stage the larva is already ensheathed (fig. 4), the sheath inclosing a short-tailed infective larva.

Infective larva.—Though the infective larva undergoes only one molt, it must be considered as the homologue of the third-stage infective larva of other Trichostrongylidae since it presents morphological features typical of third-stage larvae. In the life cycle of *L. musculi* the molt corresponding to the first molt of other strongyles is evidently suppressed, the molt which takes place being the homologue of the usual second molt since it gives rise to an infective larva.

The infective larva (fig. 5) has the general features of the first-stage larva, differing from the latter principally in the structure of the esophagus and the shape of the tail. It is 610 to 677μ long and 26μ wide. The mouth is closed and leads into a buccal cavity or pharynx about 8μ long, which in turn communicates with a club-shaped esophagus about 163 to 171μ long; the intestine, about 425μ long, is followed by a rectum about 38μ long. The nerve ring, excretory pore and genital primordium are 110μ, 121 to 129μ, and 350 to 587μ, respectively, from the anterior extremity. The tail (figs. 6 and 7) is relatively short and blunt, from 47 to 57μ long, and is provided with two subventral processes located about 10μ from its tip.

Growth of infective larva in host.—In the intestine of the host the third-stage larva increases gradually in length and in width, attaining a size of 750μ by 34μ about 24 hours after experimental infection. Evidence of the first parasitic molt was found in two larvae 725μ long by 26μ wide and 750μ long by 34μ wide, respectively, the smaller worm (fig. 8) being recognizable as a male and the larger worm (fig. 9) as a female, by the respective positions of the genital primordia, that of the female having migrated posteriorly. In the preadult stage the vulva and vagina are seen in the relative position taken up by this genital primordium.

Preadult stage.—The larvae grow considerably during this stage, and show unmistakable sex differentiation. The anterior portion of the larva (fig. 13) shows a small provisional buccal capsule and a cuticular inflation around the head extending to a distance of about 25μ posteriorly. The posterior portion of the male (fig. 10) is distended; the swollen portion forms the bursa and the indistinct folds are the precursors of the bursal rays. In the female (fig. 12) the vulva

and other accessory parts of the reproductive system, as well as the ovary, are well developed about 3 days after experimental infection. At this time the males are 1.38 to slightly over 2 mm. long by 40 to 77μ wide in the swollen posterior portion, and the females are 1.8 to 2.35 mm. long by 50 to 75μ wide. Five days after experimental infection, the preadult worms, already showing evidence of the last ecdysis, are about 3.2 to 3.4 mm. long by 78 to 83μ wide. The rays of the male bursa are fully developed in the worms undergoing the final molt (fig. 11). In a small series of measurements involving only 2 worms of each sex, the males were 3.11 to 3.4 mm. long by 78 to 93μ wide and the females were 3.2 mm. long by 76 to 83μ wide.

Young adult stage.—In young fifth-stage worms, 5 days after experimental infection, the largest females measured 5.1 mm., whereas the largest males were only 4.1 mm. long. In the male at this stage (fig. 14) the bursa and spicules have the characteristic morphology of those of the fully developed adult worm.

DISCUSSION OF LIFE HISTORY

The life history of *Longistriata musculi* presents several interesting features in its development, namely, (1) a deviation from the usual four molts which characterize the development of nematodes generally; (2) the adaptation of the infective larvae to entrance into the host through the mouth and through the skin, either avenue of infection leading to development of the worms to fertile maturity; (3) the migratory course of the larvae following skin penetration, in which the usual route through the lungs is apparently followed only exceptionally; (4) the speed with which the infective larvae reach the stomach and intestine following percutaneous infection; and (5) the failure of the larvae to undergo any evident extraintestinal development following percutaneous infection.

With regard to the number of molts involved in the life history of *L. musculi*, this case is paralleled by the development of *Nippostrongylus muris* as determined by Yokogawa (7). The latter species molts only once during its free-living existence, and the larva is infective to rats after discarding its sheath. Yokogawa regarded the infective larva of *N. muris* as a second-stage larva and considered the development of the worm in the lungs as involving 2 stages, though only one molt was present. Following the first parasitic molt in the lungs, Yokogawa regarded the exsheathed larvae as fourth-stage larvae, a view which fits their morphological status. As already indicated, the writers disagree with Yokogawa's interpretation of the

morphological status of the infective larvae and with his assumption that the growth in the lungs which culminates in a molt involves two stages, one molt being suppressed and, instead, regard the infective larva of *N. muris* as well as that of *L. musculi* as morphologically and physiologically identical with other third-stage strongyle larvae. The morphological identity is evident from the structure of the esophagus which is club-shaped and lacks a masticatory apparatus, in contrast to the rhabditiform esophagus containing a masticatory apparatus which is characteristic of second-stage as well as first-stage strongyle larvae. Moreover, the mouth of third-stage strongyle larvae is closed, whereas in the first and second stages the mouth is open. In this respect, too, the two species under consideration agree with third-stage rather than with second-stage larvae. In addition to the facts already cited, the time which elapses between the hatching of the larvae and the attainment of the infective stage, 4 days in the case of *L. musculi* and 4 to 5 days in the case of *N. muris*, lends additional support to the view that one molt has been suppressed. Under favorable conditions, strongyle larvae molt about 2 days after hatching and molt again two or three days later, the entire preparasitic development being completed in about 4 to 5 days.

From the viewpoint of their behavior, the exsheathed free-living larvae of *N. muris* and of *L. musculi* show the characteristic habits of third-stage larvae. The exsheathed larvae of both forms migrate upwards in culture dishes and bottles and are capable of infecting susceptible hosts, behavior features not exhibited by any known second-stage strongyle larvae. In the opinion of the writers, the preparasitic development of *N. muris* and *L. musculi*, which culminates in a molt, corresponds to the preparasitic development of other strongyles, the first molt being suppressed; the single ecdysis which takes place corresponds to the second molt of other strongyles. It is perhaps significant that the only two species of strongyles of which the free-living development involves only one molt, so far as known at present, are rather closely related and belong to the family Heligmosomidae. It is possible that the suppression of the first molt may be found to be a common feature in the life history of the members of this family.

Since the various stages in the development of nematodes after hatching are separated by molts, the infective larvae of *Longistriata* and *Nippostrongylus* are actually second-stage larvae having a morphology characteristic of third-stage strongyle larvae. However, in order to avoid the designation "third-stage larva" for a worm which has molted only once, the writers propose the following terms for the

stages in the development of strongyles after hatching: First preinfective larva; second preinfective larva; infective larva; preadult; adult. In the two species under discussion, the first two stages are not separated by a molt and only four stages appear after hatching, namely, (1) preinfective larva, (2) infective larva, (3) preadult, and (4) adult. The proposed designations, which have been used in this paper, have the additional advantage of eliminating the term "fourth-stage larva" for a stage in development which can no longer be regarded as larval, since sex differentiation is not only well established but is readily apparent even on superficial examination.

It is quite evident, in view of the rather ample data available on the post-mortem findings in mice at various intervals following percutaneous infection, that the larvae of *L. musculi* become arrested in the lungs only exceptionally even if they do migrate through the respiratory tract. This, as well as the probability of a more direct course of migration to the alimentary canal, accounts for the exceptionally rapid appearance of the larvae in the stomach and intestine following skin penetration. As is well known, the migratory course of various species of hookworms following percutaneous infection is from the skin to the lungs and results in a considerable delay of the larvae in these organs. The boring of the larvae through the pulmonary capillaries, their migration into and from the alveoli, along the ramifying bronchioles, up the bronchi and the trachea and thence into the esophagus, is evidently time consuming and accounts for the relatively long interval elapsing between the penetration of the larvae into the skin and their arrival in the intestine.

The essential facts in the development of *L. musculi* following the entry of the larvae through the skin are in striking contrast to those observed by Yokogawa and others with reference to the development of *N. muris*. The infective larvae of the latter species develop in the lungs, molt there, and enter the intestine as preadults. In fact the writers (4) have shown that infective larvae of *N. muris* are incapable of surviving in the digestive tract of rats, and if they fail to reach the lungs after being swallowed, they pass into the large intestine where they die and are expelled with the feces. *L. musculi*, on the other hand, undergoes its entire parasitic development in the small intestine regardless of the portal of entry into the body of its host. The ability of the infective larvae of this species to penetrate the skin is not correlated with an extraintestinal developmental phase as it is in the case of *N. muris*. The infective larvae of the latter, as a matter of fact, are not well adapted to utilizing the mouth as a portal of entry into

rats, as shown by Yokogawa (7), Africa (1) and by the writers (5). *Nippostrongylus* is a striking example among strongyles of an almost obligatory skin penetrator, since this avenue of entrance into its hosts leads to the lungs whereas an entry through the mouth results as a rule in only a slight infestation or in a failure of the worms to become established in the host.

COURSE OF INFECTION WITH L. MUSCULI

The course of infection with *L. musculi*, in so far as this can be determined by quantitative studies in the form of counts, made at more or less regular intervals, of the number of worm eggs in definite amounts of the feces of the experimentally infected white mice, was studied in 5 host animals of which 3 were infected percutaneously and 2 through the oral route. Each mouse received an initial dose of 500 larvae, and the 3 mice which were superinfected received a similar second dose. The feces of these mice were examined on the sixth day following experimental infection, with negative results in all cases. Eggs were found by the salt flotation technic on the 7th day and the counts were begun either on that day or the next day.

Figure 15 is a graphic representation of the rise and fall in the egg output of the worms in mice nos. 23, 24 and 25 which were infected through the skin. The graphs show that the peak of egg production in the case of mice nos. 23 and 24 was reached on the 9th day after experimental infection, or 2 days after eggs were first noted in the feces, and that eggs were no longer demonstrable in the feces on the 14th day in case of mouse no. 24 and on the 16th day in the case of mouse no. 23. The two mice were superinfected through the skin 18 days after the first infection.

Mouse no. 23 was kept under observation until it died, 69 days after superinfection. During this period only one egg was discovered in the feces on the 9th day and three eggs on the 15th day after superinfection; these eggs were demonstrated by the salt flotation technique. At necropsy no worms were found in the intestine of this mouse.

Mouse no. 24 began to discharge eggs 7 days after superinfection and was still discharging eggs 41 days after superinfection; two days later this mouse died and post-mortem examination showed 18 gravid females and 13 males in the small intestine.

Mouse no. 25 reached a peak of egg elimination 8 days after experimental infection and showed no eggs in the feces 5 days later. Two days after the mouse became negative it was superinfected

percutaneously. An inspection of the graph shows that the slight egg output from the worms of this mouse, beginning 9 days after superinfection, disappeared after a few days, and that following this no eggs were demonstrable in the feces for 30 days, except once as noted on the graph. This was followed by the reappearance of small numbers of eggs in the feces during a period of 15 days at the end of which, 65 days after superinfection, the mouse died. Post-mortem

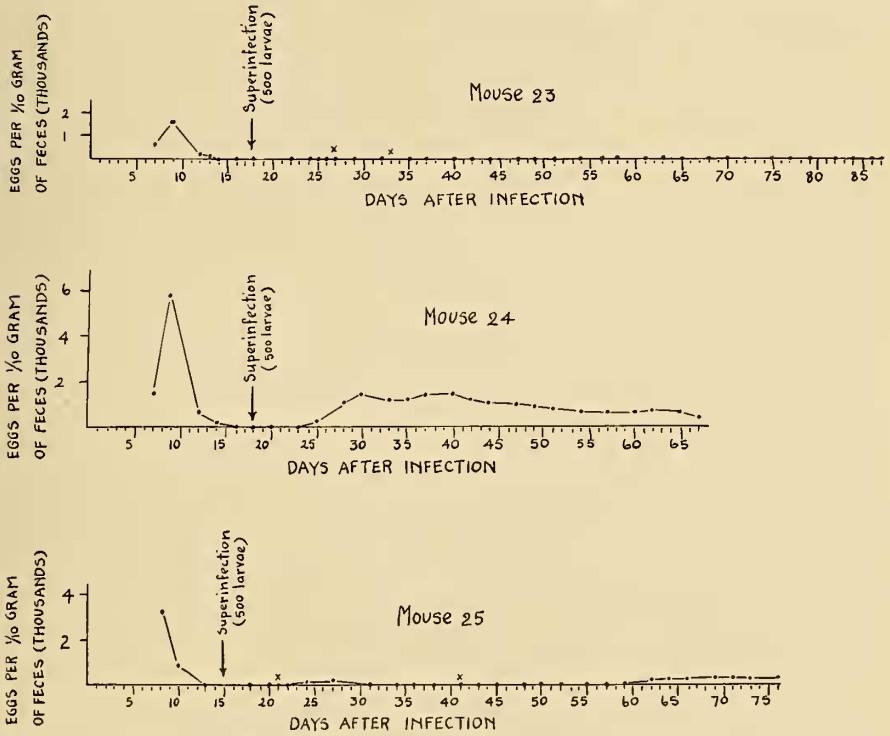

Fig. 15.—Graph of eggs per one-tenth gram of feces of mice nos. 23, 24 and 25, each infected percutaneously with 500 larvae, and superinfected percutaneously with 500 larvae as indicated. x indicates 1 to 3 eggs in total fecal output.

examination showed 22 worms in the intestine, 9 males and 13 gravid females.

From these data it is evident that following percutaneous infection of mice with *L. musculi*, the egg output quickly reached a peak and that this was followed by an equally rapid decline. A superinfection, in so far as available data show, either failed to reestablish egg production, or reestablished egg production at a level lower than that attained during the initial infection. However, the egg output following the second infection, was more stable and persisted for a rela-

tively long time. The egg output of the worms in mouse no. 25, following superinfection, involved a prolonged negative phase between 2 positive phases, due perhaps in part to a delayed development of

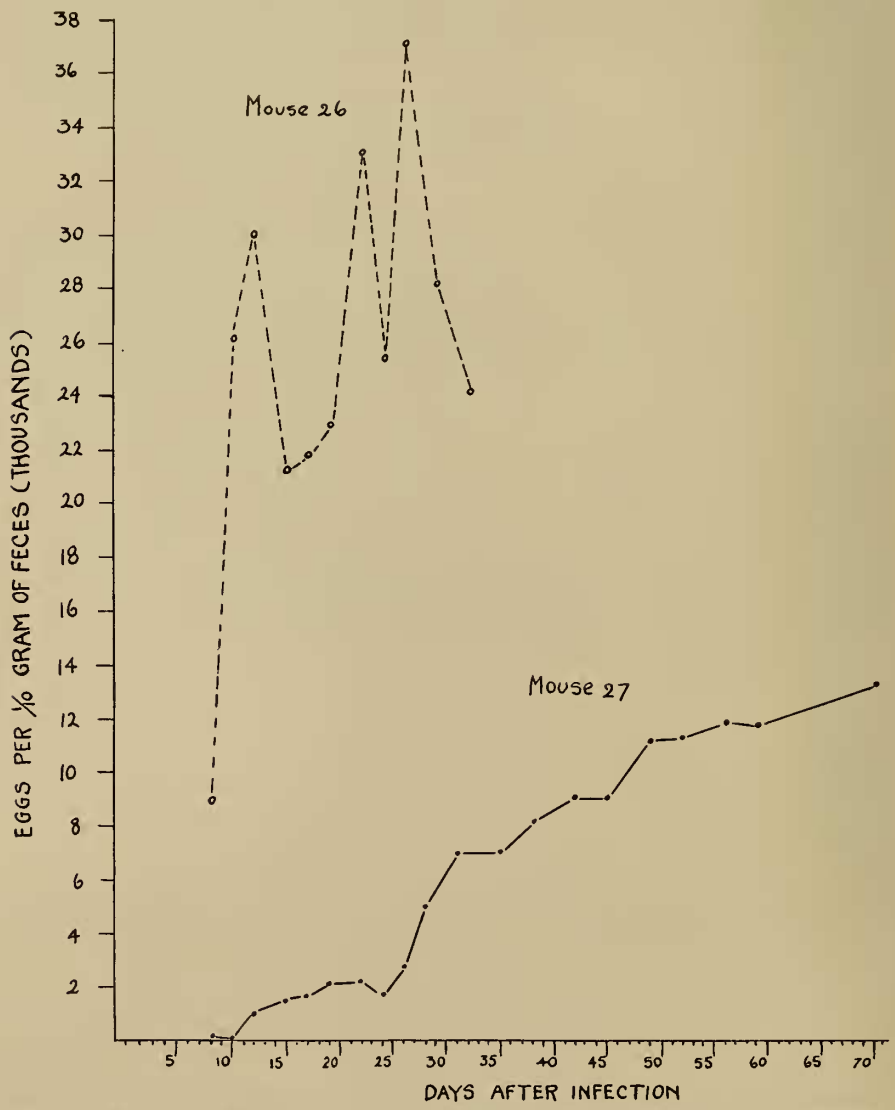

Fig. 16.—Graph of eggs per one-tenth gram of feces of mice nos. 26 and 27, each infected through the mouth with 500 larvae.

some of the worms, similar to the delayed development of *Nippostrongylus muris* following superinfection, as determined by Schwartz, Alicata, and Lucker (5), in 1931, and subsequently confirmed by Chandler (2), Spindler (6), and Graham (3).

The graphs shown in fig. 16 are of the egg output of mice nos. 26 and 27 infected through the mouth. An inspection of these graphs shows not only a tremendously large output of the eggs as compared to that of the mice infected percutaneously, but shows also a prolonged persistence in egg production at high levels. Eggs appeared in the feces of mouse no. 26 seven days after experimental infection and were still being discharged in large numbers 25 days later when the last egg count was made. Two days subsequent to the last egg count this mouse died. Post-mortem examination showed 53 worms in the small intestine, 18 males and 35 gravid females.

In mouse no. 27, infected on the same date as mouse no. 26, eggs appeared 7 days following percutaneous infection. The increase in egg output was more gradual than that in mouse no. 26. Egg production was still on the increase 63 days after experimental infection, the date on which the last count was made. Three days later the mouse died; post-mortem examination showed 103 worms in the intestine, 41 males and 62 gravid females.

It is evident from an inspection of the graphs (figs. 15 and 16) and from the data given in the text, that while eggs were first demonstrable in the feces of the mice 7 days after experimental infection, regardless of the portal of entry of the larvae, the number of eggs discharged by the worms and the duration of egg production are correlated with the portal of entry of the larvae. The percutaneous route resulted in a relatively slight egg output which lasted but a few days, whereas the entry of the larvae through the mouth resulted in a relatively tremendous output of eggs which persisted at high levels as long as the mice survived. The rapid disappearance of eggs from the feces of percutaneously infected mice can not be accounted for on the assumption of slighter infections resulting from the entry of the larvae through the skin, as compared to those resulting from the ingestion of larvae. In a series of experiments involving 5 mice (nos. 28 to 32) infected percutaneously with 300 to 500 larvae, post-mortem worm counts made from 7 to 16 days following infection, yielded 102, 158 and 86 worms, respectively, in the mice given 500 larvae each, and 47 and 55 worms, respectively, in the 2 mice given 300 larvae each, with males and females present in fairly equal numbers in all cases. These figures compare favorably with the number of worms recovered from mice nos. 26 and 27 following infection through the mouth. Assuming, therefore, that the wide discrepancy in the number of eggs produced by the worms following the two avenues of entrance into the host are not due to differences in the percentage of

larvae which actually reached the intestine and developed there to maturity, it is probable that the migration of the larvae from the skin to the intestine, involving a passage through various tissues and cavities, stimulated the defense mechanism of the body. The response to this stimulation is apparently of a sort which interferes with egg production even before the worms die and are eliminated from the intestine. The amazingly low egg output from the worms in mice nos. 24 and 25, despite the presence of 18 and 13 female worms, respectively, in these two animals, as compared to the egg output of the worms in mouse no. 26 which had approximately only twice as many females, or even as compared with the egg output of the worms in mouse no. 27 which harbored 62 females, is certainly suggestive of a host resistance involving among other things inhibition of egg production.

In the case of *N. muris*, the inhibition of development and of egg production has been confirmed by several workers, as already stated, since Schwartz, Alicata and Lucker (5) called attention to this fact. Experimental percutaneous infection of rats with *Nippostrongylus*, as determined by these workers, resulted in most cases in the rapid attainment of a peak in egg production followed, as a rule, by an equally rapid decline. In superinfections, produced following this decline, but few or no eggs were demonstrable in the feces of a large proportion of rats, despite the presence in the intestine of relatively large numbers of worms, including gravid females. The course of infection with *Nippostrongylus* in rats following the invasion by larvae through the skin is similar, as a rule, to the course of infection with *Longistriata* in mice following the same portal of entry. This general similarity in egg production coupled with the same avenue of entrance into the body, suggests that the passage of the larvae of the two species under discussion through the tissues of their respective hosts brings about a defense reaction to the invasion of the parasites which terminates the egg production and, therefore, the multiplicative capacity of the worms, in a few days.

SUMMARY

Under favorable conditions, the eggs of *Longistriata musculi* hatched in about 24 hours after they were eliminated from the host, *Mus musculus*, and the larvae attained their full development in 4 days. Following one preparasitic molt, the larvae were infective to mice.

Although the infective larva has molted only once, its morphology

and behavior are similar to known third-stage trichostrongyle larvae. The view is advanced that the first molt has been suppressed, and the molt which takes place corresponds to the second preparasitic molt of related nematodes. As established by visible molts, it is a second-stage larva, but, as established by morphology and behavior, it is the equivalent of the infective third-stage larva of trichostrongyles in general.

The following designations are proposed in this paper for the stages in the development of strongyles: (1) First preinfective larva; (2) second preinfective larva; (3) infective larva; (4) preadult; and (5) adult. The suppression of one molt during the free-living period reduces the life cycle to 4 stages.

White mice were infected with *Longistriata* through the mouth and through the skin, either portal of entry leading the worms to the small intestine, where they undergo their entire development, accompanied by two molts.

A few hours after percutaneous infection, larvae were found in the stomach and intestine and they became localized in the intestine exclusively 24 hours after having been placed on the skin.

The precise route taken by the larvae from the skin to the intestine has not been determined; evidently, the migratory course usually followed by skin-penetrating nematodes, involving a passage through the lungs, was followed only exceptionally by *L. musculi*, so far as available data show.

Preadult worms, showing unmistakable sex differentiation, were found in the intestine of white mice about 48 hours after experimental infection through the mouth or skin, and final stage worms (adults), not fully grown, were found in these host animals 5 days after entry by either portal.

Regardless of the portal of entry of the larvae, eggs were first noted in the feces of experimentally infected mice 7 days after the administration of larvae.

The period of egg production in 3 white mice infected percutaneously with 500 larvae was limited to approximately two weeks. Superinfection with 500 larvae following the apparent cessation of egg production, yielded practically negative results in one case coupled with absence of worms in the intestine, and resulted in only a small output of eggs in the two remaining mice which harbored worms of both sexes, the egg output being far below the expected output, considering the number of females present.

Following infection with 500 larvae through the mouth, the egg

output from 2 mice reached a far higher level than that attained following percutaneous infection. Moreover, the high level of egg production persisted until the mice died, 32 and 63 days, respectively, following the ingestion of larvae.

It is suggested that the glaring differences in egg production by the worms, the differences correlated with the portal of entry of the larvae into white mice, is probably due to a marked stimulation of the defense mechanism of the host coincident with the migration of the larvae through various tissues following percutaneous infections. This stimulation is either lacking or is not marked following ingestion of larvae.

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